SUPPORT FOR AMENDMENTS

This amendment contains no new matter. Applicants have amended the claims to be consonant with the second restriction requirement of November 20, 2002. Support for the amended claims can be found on p.10 line 27 to p.11, line 1; p. 22, line 24 to p. 23, line 3; and claims 47-51 of the originally-filed application. Claims 57-66 are pending in the application.

REQUEST FOR RECONSIDERATION

Applicants would like to thank Examiner Bunner for her helpful suggestion for the change of title.

Pluripotent stem cells differentiate to many different cell types. For example, stem cells harbored in bone marrow give rise to hematopoietic cells, such as eosinophils, basophils, neutrophils, megakaryoctes (giving rise to platelets), and erthryocytes. A major problem impeding progress in stem cell therapies is the accumulation of pure cultures of stem cells. However, inhibiting differentiation of a stem cell confers the advantage of accumulating many stem cells in a pure culture *in lieu* of cultures having various differentiated populations that arise spontaneously *in vitro*. Such an accumulation of undifferentiated stem cells can be especially beneficial in cell replacement therapies, thus providing a generous source of cells ready for transplanting. Such an approach can be further exploited, for example, by using regenerative stem cells found in adult tissues that have the potential to differentiate into hematopoietic stem, muscle, liver and pancreas cells. Inhibiting stem cells from differentiating better controls their response to differentiating factors, enabling enriched cultures of newly differentiated cell types. Inhibiting cell differentiation can also be used to *direct* cell differentiation. For example, stem cells can be activated to pursue various avenues of differentiation by either extracellular factors alone or in combination with timing (the age of the cell). Inhibiting stem cell differentiation can be used to unravel the complexities of hematopoiesis. Finally, a population of stem cells may be synchronized not only at the level of the cell cycle, but now also at the level of differentiation.

The claimed invention solves the problems of controlling stem cell differentiation by inhibiting stem cell differentiation, allowing for the preparation of large pure stem cell cultures, using the polypeptide HEMA2 (SEQ ID NO:4).

Claim Rejections 35 USC §112, first paragraph

The rejection of claims 57-66 under 35 USC § 112, first paragraph, is respectfully traversed. The specification teaches how to use the HEMA polypeptides (subsequently referred to as HEMAs) to inhibit hematopoietic cell differentiation; furthermore, one of skill in the art would know how to use the invention because art published before August 19, 1999 teach using polypeptides to inhibit hematopoietic stem cell differentiation, both *in vitro* and *in vivo*.

The specification teaches how to use the HEMAs to inhibit hematopoietic cell differentiation. First, the specification teaches that HEMAs can be used to inhibit hematopoietic

stem cell differentiation (page 22, lines 24-25). Secondly, the specification teaches that the "expression of HEMA sequences similar to the HEMA expression pattern of DAS 104-4 indicates that the cell population has a hematopoietic status that supports the proliferation, *i.e.*, self renewal [*sic*] of hematopoietic stems [*sic*] cells" (page 17, lines 8-10). Stem cells that self-renew are stem cells that do not differentiate.

To use the invention, one contacts a cell with the protein. An inhibitory amount is the amount of HEMA2 which is found in this culture of DAS 104-4 cells where the stem cells do not differentiate and can be easily determined. Using antibodies or other protein quantification techniques, all well known in the art, the amount of HEMA2 can be quantified and calculated to be expressed as amount per cell, per mass, *etc.*. Accordingly, the specification provides sufficient guidance to make and use the invention. See MPEP 2164.06 (b) "SEVERAL DECISIONS RULING THAT THE DISCLOSURE WAS ENABLING" (C), citing *In re Bundy*, 209 USPQ 48, 51-52 (CCPA 1981).

The literature provides ample examples of using polypeptides to affect hematopoietic cell function. Large polypeptides, such as antibodies and cell polypeptides, can be used to inhibit hematopoietic cell differentiation and proliferation *in vitro*. Lecomte-Raclet *et al.* (1998) describe the active domains of platelet factor 4 (PF4) which inhibit activity towards hematopoietic progenitor cells (p. 2772, 2nd column, end of paragraph preceding "Materials and Methods"). Using $F(ab')^2$ fragments of anti-PF4 and anti-active peptide antibodies, Lecomte-Raclet *et al.* were able to inhibit the inhibitory effects of their antigens (p. 2776, "Effect of Various Anti-PF4 Antibodies on CFU-MK Growth;" p. 2778, Fig. 7).

Su *et al.* (1997) were also able to use antibodies to reverse inhibitory effects of a chemokine ligand, MIP-1α. Figure 5 (p. 609) shows that 100 μg/ml of anti-MIP-1α polyclonal antibody mostly reversed the effects of MIP-1α on burst-forming unit-erythroid (BFU-E) formation.

Large polypeptides, such as antibodies and cell polypeptides, can be used *in vivo*. Ishida *et al.* (1998), investigating Langerhans cell function (as a model for dendritic cell function) in tumorigenesis, injected VEGF-producing D459 cells into mice to induce tumors. After the tumors had grown to 5 to 6 mm in diameter, mice were injected with 10 μg/mouse of anti-VEGF polyclonal antibody twice a week for four weeks; after which the presence of dendritic cells was examined. The antibody enabled an increase in the number of dendritic cells compared to

controls (p. 4847, "Effect of neutralizing anti-VEGF Ab on LC function *in vivo*"; p. 4850, Figure 8).

Small polypeptides, such as HEMAs and peptides, can be used to inhibit hematopoietic cell differentiation and proliferation *in vitro*. Lecomte-Raclet *et al.* (1998) purified PF4, and synthesized related peptides (p. 2773, "PF4 Purification" and "Peptide Synthesis"). Using colony assays of hematopoietic progenitors (p. 2773, "Colony Assays of Hematopoietic Progenitors"), Lecomte-Raclet *et al.* applied 130 nmol/L (1 μg/ml) or more of PF4 and 2.2 nmol/L or more of the active peptides to inhibit megakaryocytopoiesis (platelet formation) *in vitro* (p. 2774, "Results," 2nd paragraph, Fig. 2; p. 2775, Fig. 3, Fig. 4 and Table 1). When colony formation from enriched $CD34^+$ cell cultures was examined in the presence of PF4 and its derived peptides, colony formation was also inhibited (p. 2776, Fig. 5). In this instance, the same concentrations of PF4 and the active peptide were the same (130 nmol/L and 2.2 nmol/L, respectively to inhibit megakaryocytopoiesis), whereas inhibition of granulocyte-macrophage and erythroid cells occurred at 650 nM/L of PF4. The peptides in all cases required only 2.2 nmol/L. Similar results using the chemokine receptor ligand MIP-1α, a small molecular mass polypeptide, were obtained by Su *et al.* (1997). They found maximal inhibition of erythroid cell colony formation at 100 ng/ml (100 μg/L) (p. 607, second column; p. 608, Fig. 3B).

Small polypeptides, such as HEMAs and peptides, can be used to inhibit hematopoietic cell differentiation and proliferation *in vivo*. Veiby *et al.* (1996) irradiated syngeneic mice and transplanted hematopoietic stem cells (phenotype Lin^-Sca1^+). Those mice that had been injected with the SL&F108636 peptide (0.1 ng - 10 ng/kg) daily for 11 days after transplantation had a 50% reduction in the production/differentiation of various cells (p. 220, 2nd column; p.221, Table 2; p. 222, 1st column, 1st full paragraph). One-tenth of a nanogram per kilogram was sufficient to achieve this result (Table 2).

The Office has also raised the concern that insufficient guidance has been provided to enable the use of polypeptides having at least 80% sequence identity to SEQ ID NO:4, without undue experimentation. Applicants respectfully disagree.

Many substitutions of amino acids in a polypeptide can be made without impairing the function of the polypeptide, although certain substitutions in certain critical areas may impair function, as noted in the Office Action. However, by keeping at least 80% sequence identity to SEQ ID NO:4, these function-impairing substitution are expect to be relatively rare in comparison to those substitution which do not impair function, and even those substitutions that

impair function usually do not destroy it, rather they simply reduce the activity (see, for example, Bowie *et al.* (1990)). Guidance has been provided to aid in the selection of changes which are likely not to effect function (for example, see line page 35, line 18 to page 36 line 19 (chimeric and fusion polypeptides comprising the polypeptide sequence of SEQ ID NO:4) and page 31, line 36 to page 32, line 15 for variants wherein conservative amino acid substitutions have been made).

Finally, even the rare cases that might result in loss of activity do not require undue experimentation to identify. The presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled; the standard is whether a skilled person could determine which embodiments would be inoperative or operative with expenditure of no more effort than is normally required in the art (MPEP § 2164.08(b), citing *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 224 USPQ 409, 414 (Fed. Cir. 1984)). Determining if a chosen polypeptide is active merely requires applying it to the stem cell culture and examining the culture for differentiation; this type of experimentation is routine in the art, and is not unusually time consuming or expensive in the art. Compare this to the selection of monoclonal hybridomas to determine which ones secrete antibody with the desired characteristics, where the Court concluded that undue experimentation would not be required (MPEP 2164.06 (b) "SEVERAL DECISIONS RULING THAT THE DISCLOSURE WAS ENABLING" (B), citing *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988)).

Accordingly, applicants submit that the claimed invention is enabled, without undue experimentation. Withdrawal of this ground of rejection is respectfully requested.

Applicants request the Office to reconsider the rejection of claims 65 and 66 under 35 USC § 112, second paragraph. Antecedent basis for "stem cell" in both claims is found in claim 63, which depends from claim 57, which recites "a stem cell" in the preamble.

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